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[Continued on next page]

(54) Title: ACTINOBACILLUS PLEUROPNEUMONIAE VIRULENCE GENES

Class	Sheet	Draw	Sheet/Draw	%	Span	Number	Name or Positive Position	In vitro CD	In vitro CD
Cell Culture	8C3	sp4C					AAC20191	cluster polysaccharide repeat	
	250A8		10B (10a)	27D(2)	12		AAP7181	LPS or capsule Mucopenta	1.000
	937	sp4U					ANC20192	LPS core Mucopenta	1.250
	2387	sp4U					ANQ4894	LPS O-antigen Mucopenta	0.0244
	102811	sp4C	10a	13			AAC48941	LPS O-antigen Mucopenta	0.233
	1226	sp4U					AAC48942	LPS O-antigen Mucopenta	0.024
	2138	sp4P					AAC48943	LPS O-antigen Mucopenta	0.023
	1248	sp4C	PCT03 170	27D(2)	276		U014871170	LPS O-antigen Mucopenta	0.239
	404	sp4P	48D(2)	89			AAP20208	Spemann B protein	1.000
	1741	sp4P	48D(2)	227			ANQ20209	Galactosidase protein P4	1.149
Microbiol	10212	sp4P	81D(2)	63			P44221	Protein	1.000
	10411	sp4P	65D(2)	34			AAC22373	mannosidase sulphatase	0.002
	3327	sp4A	88D(2)	134			P44234	ATP sulphatase	0.214
	1786	sp4P	52D(2)	168			P44235	spemann C antigenic protein	1.100
	24011	sp4P	62D(2)	160			PS1510	mannosidase sulphatase	1.000
	28C3	sp4C	61D(2)	394			CAU102	Galactosidase inhibitor	0.002
	439	sp4C	67D(2)	114			P44211	mannosidase sulphatase protein	1.100
	1100	170					AAC0188	ATPase	0.020
	25011	sp4P	58D(2)	77			AAC20261	peptidase alpha inhibitor	1.000
	985	sp4P	78D(2)	123			AAP4941	peptidase alpha phosphopeptidase	1.100
	047	sp4D	56D(2)	428			P44219	MAP65	0.750
	2320	sp4C	PCT03 170				ANQ20219	heat-stabilizing MAP65	0.750
	2384	sp4C	72D(2)	345			AAC20220	alpha-1 antitrypsin	0.750
	2385	sp4C	61D(2)	71			P44221	peptidase alpha inhibitor	0.002
	238410	sp4C	68D(2)	168			P44232	Therapeptidase	0.007
	078	sp4C	84D(2)	238			PS1510	Energy regulator	0.400
	27A12	sp4C					Y1710	Energy regulator	0.300
	8004	sp4C	88D(2)	221			ANQ20210	Urease inhibitor	0.001
	12411	sp4C	52D(2)	43			AAC20220	alpha-1 antitrypsin	0.004
	2844	sp4C	58D(2)	127			P44220	peptidase alpha inhibitor	0.000
	2387	sp4C	58D(2)	167			P44220	peptidase alpha inhibitor	0.744
	30012	sp4C	62D(2)	43			P44220	GMP synthase	1.000
	0212	sp4C	78D(2)	120			P44223	beta-1,3-glucanase	0.027
	2802	sp4C	68D(2)	119			P44227	beta-1,3-glucanase	0.003
	12510	sp4C	42D(2)	61			AAC20287	negative regulator of cycl	1.725
	21320	sp4C	51D(2)	160			P44220	Energy regulator	0.700

(57) Abstract: An attenuated *Actinobacillus pleuropneumoniae* bacterium has a mutation in a gene required for bacterial virulence. Vaccines based upon the bacterium are provided, as are isolated virulence genes and polypeptides and uses thereof.



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4 November 2004

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 03/05349

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/31 C07K14/285 C07K16/12 C12N5/10 G01N33/50
A61K39/102

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07K G01N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>WO 00/61724 A (PHARMACIA & UPJOHN, INC.) 19 October 2000 (2000-10-19) cited in the application the whole document see especially: SEQ ID NOS: 140, 141 ORF ID: exbB mutant ID: AP11E7 page 42 - page 53; examples 7-11; tables 2-4 and page 233 - page 234</p> <p>-----</p> <p style="text-align: center;">-/-</p>	1-13, 16-35

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the International filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the International filing date but later than the priority date claimed

- "T" later document published after the International filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the International search

21 June 2004

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14 09 2004

Name and mailing address of the ISA

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INTERNATIONAL SEARCH REPORT

Inte nal Application No
PCT/GB 03/05349

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 02/075507 A (PHARMACIA & UPJOHN COMPANY) 26 September 2002 (2002-09-26) the whole document see especially: SEQ ID NO: 140, 141 ORF ID: exbB mutant ID: AP11E7 page 45 - page 57; examples 7-11; tables 2-4 and page 234 - page 236 -----	1-13, 16-35
X	ELKINS, C. ET AL.: "Role of the Haemophilus ducreyi Ton System in Internalization of Heme from Hemoglobin" INFECTATION AND IMMUNITY, vol. 66, no. 1, January 1998 (1998-01), pages 151-160, XP002285338 the whole document	16-27, 30-33
A	see especially: page 154 - page 155; figure 2 ExbB, ExbD, TonB proteins and page 158, column 2, line 26 - line 37 -----	1-13, 28, 29, 34, 35
A	FULLER, T.E. ET AL.: "A genetically-defined riboflavin auxotroph of <i>Actinobacillus pleuropneumoniae</i> as a live attenuated vaccine" VACCINE, vol. 18, no. 25, 15 June 2000 (2000-06-15), pages 2867-2877, XP004203577 the whole document -----	1-13, 16-35
T	BEDDEK, A.J. ET AL.: "Two TonB Systems in <i>Actinobacillus pleuropneumoniae</i> : Their Roles in Iron Acquisition and Virulence" INFECTATION AND IMMUNITY, vol. 72, no. 2, February 2004 (2004-02), pages 701-708, XP008031967 the whole document -----	1-13, 16-35

INTERNATIONAL SEARCH REPORT

national application No.
PCT/GB 03/05349

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:

2. Claims Nos.: 14, 15, 36-40 (completely) and 17, 18, 29-33 (partially) because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210

3. Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-13, 16-35 (partially)

Remark on Protest

The additional search fees were accompanied by the applicant's protest.
 No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 14, 15, 36-40 (completely) and 17, 18, 29-33 (partially)

Present claims 14 and 15 and dependent claims 17, 18, 29-33 relate to a polynucleotide defined by reference to a desirable characteristic or property, namely, for claim 14: "encoding a gene product which is not naturally found in *A. pleuropneumoniae*, but whose expression therein is capable of modulating ... the virulence of that bacterium", and for claim 15: "which is not naturally found in *A. pleuropneumoniae* but which is capable of modulating the virulence of that bacterium by its direct interaction with *A. pleuropneumoniae* virulence genes or gene products".

The claims cover all polynucleotides having this characteristic or property, whereas the application provides no support within the meaning of Article 6 PCT and no disclosure within the meaning of Article 5 PCT for such polynucleotides. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the polynucleotide by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search impossible. Consequently, no search has been carried out for claims 14 and 15 and for dependent claims 17, 18, 29-33.

Furthermore, present claims 36, 38-40 relate to an anti-bacterial agent defined by reference to a desirable characteristic or property, namely "identified by the method of claims 34 or 35".

The claims cover all compounds having this characteristic or property, whereas the application provides no support within the meaning of Article 6 PCT and no disclosure within the meaning of Article 5 PCT for such compounds. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the compound by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search impossible. Consequently, no search has been carried out for claims 36, 38-40.

The same applies to claim 37 relating to a "method of modulating the transcription of such virulence genes through the use of oligonucleotide-directed triplet helix formation". However, the application provides no support within the meaning of Article 6 PCT and no disclosure within the meaning of Article 5 PCT for such oligonucleotides. Accordingly, no search has been carried out for claim 37.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

search report has been established need not to be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guideline C-VI, 8.5), should the problems which led to the Article 17(2) declaration be overcome.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-13, 16-35 (partially)

An attenuated *Actinobacillus pleuropneumoniae* bacterium having a mutation in a gene required for bacterial virulence which comprises the nucleotide sequence of SEQ ID NO: 1, a composition containing said attenuated *A. pleuropneumoniae* bacterium, use of said attenuated *A. pleuropneumoniae* bacterium in the manufacture of a medicament for preventing or alleviating an infection of an animal with *A. pleuropneumoniae*, an isolated polynucleotide comprising a) a nucleotide sequence of SEQ ID NO: 1, b) a nucleotide sequence encoding the polypeptide which is encoded by the nucleotide sequence recited in a), c) a nucleotide sequence which hybridizes to the nucleotide sequence of a) and/or b) or to its complement under conditions of moderate to high stringency, d) a fragment of any one of the nucleotide sequences of a)-c) which fragment retains an immunological properties and/or biological activity of the recited nucleotide sequence, a vector comprising said polynucleotide, a host cell comprising said vector, an isolated *A. pleuropneumoniae* polypeptide encoded by said polynucleotide, a method of producing said polypeptide, a composition containing said polypeptide, an antibody which specifically recognizes said polynucleotide or said polypeptide, a method for identifying an anti-bacterial agent which is capable of modulating the function of the said *A. pleuropneumoniae* virulence gene, an attenuated bacterium containing a mutation in a gene comprising a nucleotide sequence which is capable of hybridising to the nucleotide sequence defined by SEQ ID NO: 1 under conditions of moderate to high stringency, a composition containing said attenuated bacterium, use of said attenuated bacterium in the manufacture of a medicament for the therapeutic treatment or prophylactic protection of an animal against infection by the corresponding wild-type bacterium, an isolated virulence polypeptide encoded by said gene, a composition containing said polypeptide, an antibody which specifically recognizes said polynucleotide or said polypeptide;

2. claims: 1-13, 16-35 (partially)

idem as subject 1, but limited to SEQ ID NO: 2;

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 03/05349

Patent document cited in search report	Publication date		Patent family member(s)	Publication date
WO 0061724	A 19-10-2000		AU 4077600 A BR 0009663 A CA 2366520 A1 CN 1351653 T EP 1171577 A2 JP 2002541790 T NZ 514883 A WO 0061724 A2 US 2004110268 A1 ZA 200108262 A	14-11-2000 09-04-2002 19-10-2000 29-05-2002 16-01-2002 10-12-2002 26-03-2004 19-10-2000 10-06-2004 08-01-2003
WO 02075507	A 26-09-2002		US 2004110268 A1 CA 2438315 A1 EP 1368456 A2 WO 02075507 A2	10-06-2004 26-09-2002 10-12-2003 26-09-2002